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Colleen Coyne
Printed name of person mailing correspondence

Colleen Coyne
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Jack W. Szostak et al.	Art Unit:	
Serial No.:	09/876,235	Examiner:	
Filed:	June 6, 2001	Customer No.:	21559
Title:	SELECTION OF PROTEINS USING RNA-PROTEIN FUSIONS		

Assistant Commissioner For Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination, kindly amend the above-referenced application as follows.

In the Specification:

At page 18, replace the second paragraph (lines 16-23) with the following amended paragraph rewritten in clean form:

FIGURE 17 is a photograph illustrating the translation of myc RNA templates. The following linkers were used: lanes 1-4, $dA_{27}dCdCP$ (SEQ ID NO: 8); lanes 5-8, $dA_{27}rCrCP$ (SEQ ID NO: 8); and lanes 9-12, $dA_{21}C_9C_9C_9dAdCdCP$. In each lane, the concentration of RNA template was 600 nM, and ^{35}S-Met was used for labeling. Reaction conditions were as follows: lanes 1, 5, and 9, 30°C for 1 hour; lanes 2, 6, and 10, 30°C for 2 hours; lane 3, 7, and 11, 30°C for 1 hour, -20°C for 16 hours; and lanes 4, 8, and 12, 30°C for 1 hour, -20°C for 16 hours with 50 mM Mg^{2+}. In this Figure, "A" represents free peptide, and "B" represent mRNA-peptide fusion.

At page 19, replace the first full paragraph (lines 3-7) with the following amended paragraph rewritten in clean form:

> FIGURE 19 is a photograph illustrating the translation of myc RNA template using lysate obtained from Ambion (lane 1), Novagen (lane 2), and Amersham (lane 3). The linker utilized was $dA_{27}dCdCP$ (SEQ ID NO: 8). The concentration of the template was 600 nM, and ^{35}S-Met was used for labeling. Translations were performed at 30°C for 1 hour, and incubations were carried out at -20°C overnight in the presence of 50 mM Mg^{2+}.

At page 58, replace the third partial paragraph (lines 19-28) with the following amended paragraph rewritten in clean form:

> Using the above conditions, mRNA-puromycin conjugates were synthesized as follows. Ligation of the myc RNA sequence (RNA124) to the puromycin-containing oligonucleotide was performed using either a standard DNA splint (e.g., 5'-TTTTTTTTTTAGCGCAAGA) (SEQ ID NO: 28) or a splint containing a random base (N) at the ligation junction (e.g., 5'-TTTTTTTTTTNAGCGCAAGA) (SEQ ID NO: 33). The reactions consisted of mRNA, the DNA splint, and the puromycin oligonucleotide in a molar ratio of 1.0 : 1.5-2.0 : 1.0. An alternative molar ratio of 1.0 : 1.2 : 1.4 may also be utilized. A mixture of these components was first heated at 94°C for 1 minute and then cooled on ice for 15 minutes. Ligation reactions were performed for one hour at room temperature in 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 1 mM

Insert the enclosed Sequence Listing consisting of 9 pages at the end of the present application.

In the Claims:

Cancel claims 2-23 and add new claims 24-46.

24. (New) A method for pausing a ribosome during translation of an RNA template, said method comprising including a non-RNA moiety in said RNA template, said non-RNA moiety causing said ribosome to slow or stop its rate of translation.

25. (New) The method of claim 24, wherein said non-RNA moiety is DNA.

26. (New) The method of claim 25, wherein said DNA forms an RNA-DNA junction.

27. (New) The method of claim 24, wherein said non-RNA moiety comprises an oligo dA sequence.

28. (New) The method of claim 24, wherein said non-RNA moiety is a combination of DNA and a non-nucleotide moiety.

29. (New) The method of claim 28, wherein said non-nucleotide moiety comprises one or more $HO(CH_2CH_2O)_3PO_2$ (polyethylene glycol phosphate) moieties.

30. (New) The method of claim 24, wherein said non-RNA moiety terminates in a dCdC sequence.

31. (New) The method of claim 24, wherein said non-RNA moiety is located downstream from a protein coding sequence and wherein during said pausing of said ribosome during translation, the nascent peptide generated during translation is linked covalently to said RNA template.

32. (New) A protein-encoding RNA, said RNA molecules being covalently bonded at the 3' end of the protein coding sequence to a non-RNA pause sequence.

33. (New) The protein-encoding RNA of claim 32, wherein said non-RNA pause sequence is DNA.

34. (New) The protein-encoding RNA of claim 33, wherein said DNA forms an RNA-DNA junction.

35. (New) The protein-encoding RNA of claim 32, wherein said non-RNA pause sequence comprises an oligo dA sequence.

36. (New) The protein-encoding RNA of claim 32, wherein said non-RNA pause sequence is a combination of DNA and a non-nucleotide moiety.

37. (New) The protein-encoding RNA of claim 36, wherein said non-nucleotide moiety comprises one or more $HO(CH_2CH_2O)_3PO_2$ (polyethylene glycol phosphate) moieties.

38. (New) The protein-encoding RNA of claim 32, wherein said non-RNA pause sequence terminates in a dCdC sequence.

39. (New) The protein-encoding RNA of claim 32, wherein said protein coding sequence comprises a partially or fully randomized region.

40. (New) The protein-encoding RNA of claim 32, wherein said protein coding sequence encodes an antibody.

41. (New) The protein-encoding RNA of claim 32, wherein said protein coding sequence encodes a binding protein.

42. (New) The protein-encoding RNA of claim 41, wherein said binding protein

is a ligand binding protein.

43. (New) The protein encoding RNA of claim 32, wherein said protein coding sequence encodes an enzyme.

44. (New) The protein-encoding RNA of claim 32, wherein said protein coding sequence encodes a catalytic protein.

45. (New) The protein-encoding RNA of claim 32, wherein said RNA is messenger RNA.

46. (New) The protein-encoding RNA of claim 32, wherein said protein-encoding RNA is immobilized on a solid support.

REMARKS

Applicants have added new claims 24-46 directed to methods for pausing a ribosome during translation of an RNA template and RNA molecules that are used to accomplish such pausing. These claims find support in the specification, for example, as follows: claims 24 and 32 at page 10, lines 1-2, page 20, lines 2-5, and page 30, lines 6-9 and 23-28; claims 25, 27, 33, and 35 at page 30, line 23 and Figure 1B; claims 26 and 34

at Figure 1B; claims 28, 29, 36, and 37 at page 62, lines 20-24; claims 30 and 38 at page 30, line 23; claim 31 at page 19, line 25 through page 20, line 5; claim 39; pages 21-23; claim 40, pages 78-79; claims 41-42, page 78, lines 15-22; claims 43 and 44, pages 79-80; claim 45, page 2, lines 13-15; and claim 46, pages 81-82. In addition, the specification has been amended to correct sequence identification number omissions and errors. No new matter is added by any these amendments. Enclosed is a check for $1794.00 for the required fee.

By this amendment, applicants have also inserted the enclosed Sequence Listing at the end of the application. The paper copy of the Sequence Listing submitted herewith is identical to that filed in connection with parent application, U.S.S.N. 09/247,190, on February 15, 2000.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 19 February 2002

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Marked Up Version of Amendments

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